THE PERMEABILITY OF THE ERYTHROCYTE-LIKE CELLS OF PHASCOLOSOMA GOULDI [1]

WARNER E. LOVE

The Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa., and The Marine Biological Laboratory, Woods Hole, Mass.

The coelomic fluid of the annelid worm *Phascolosoma gouldi* contains cells of unknown function which at least superficially resemble vertebrate erythrocytes (Cuénot, 1891; Dawson, 1933). Like the latter, they have two special properties which permit the use of Jacobs' hemolysis method (1934) to determine their permeability constants. First, they lose their red pigment, hemerythrin, on swelling osmotically to a certain critical volume; the accompanying optical changes are as striking as those seen in the hemolysis of vertebrate erythrocytes. Second, their discoidal shape enables one to assume that the cell surface area does not change significantly during moderate osmotic swelling.

This paper reports the application of Jacobs' method to Phascolosoma "erythrocytes." Measurements have been made of (1) normal cell volume, (2) "hemolytic" volume, (3) cell surface area, and (4) "hemolysis" times in water and various solutions isosmotic with sea water. From these data, permeability constants have been calculated and compared with those of certain representative cells for which quantitative data exist.

MATERIAL AND METHODS

To make stock suspensions of Phascolosoma erythrocytes, coelomic fluid from ten to twenty worms was diluted to about 100 cc. with filtered sea water, which Chapeau (1928) has reported to be isotonic. Filtration through a loose plug of absorbent cotton removed the large eggs and the clot which usually formed. Repeated gentle centrifugation served to separate the erythrocytes from the much smaller sperm cells which remained suspended. The packed cells from the final centrifugation were used as the stock suspension.

Normal cell volume was determined from 8 hematocrits combined with 5 cell counts of the same suspension. The average cell volume was 1050 μ^3.

It was obviously impossible to measure accurately the hemolytic volume during hemolysis. Instead, it was estimated from microscopic measurements of the diameters of intact spherical cells remaining in the dilute sea water which previously had been found to produce 50% hemolysis. The mean diameter of fifty such cells was 14.5 μ with a standard deviation of 1.8 μ; thus the average hemolytic volume was 1610 μ^3, and the average surface area was 660 μ^2, assuming constancy during swelling. The swollen cell volume, calculated for the 60% sea water used above by the van't Hoff law from the normal cell volume, exceeds the measured hemolytic volume by an amount corresponding to a non-solvent space of 20% of the cell.

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[1] These studies were supported by a grant from The National Science Foundation.

This "dead-space" is higher than that of 7–14% for Arbacia eggs found by McCutcheon *et al.* (1931) and lower than that of about 30% reported by Ponder (1948) for mammalian erythrocytes. Although it is recognized that there may conceivably be a selective effect of this procedure for estimating hemolytic volume, the method has been used because of convenience. Its use is supported by the fact that the non-osmotic volume so estimated is in reasonable agreement with the high hemerythrin content of these cells (Florkin, 1933).

The non-electrolyte solutions were made one molar, which is osmotically roughly equivalent to Woods Hole sea water, and were lightly buffered to approximately the pH of sea water (by addition of 5% by volume of 0.33 M Na_2HPO_4 which had been brought to pH 7.8 with concentrated HCl). As a result, the pH remained essentially constant during hemolysis of added cells. One-half molar solutions of ammonium salts of fatty acids were prepared by titrating a small quantity of the proper undiluted fatty acid with $M/2$ NH_4OH to a pH such that equal amounts of ammonia and fatty acid were present in the solutions (see, in this connection, Stewart, 1934). To measure a hemolysis time in one of these solutions, 0.05 cc. of the stock suspension of cells was mixed with 10 cc. of the solution; the time of 50% hemolysis was determined by the "slit" method of Jacobs *et al.* (1949). Control experiments showed that none of the substances discussed below were hemolytic *per se*.

Results and Discussion

The observed hemolysis times are listed in the second column of Table I. In the third column are listed the corresponding permeability constants, calculated by the method of Jacobs (1934) from hemolysis times, hemolytic volume, and normal cell volume and surface area. For comparison the permeability constants of unfertilized Arbacia eggs (Jacobs and Stewart, 1932), those of cells of *Chara ceratophylla* (Collander and Bärlund, 1933) and those of beef erythrocytes (Jacobs, 1952) are also given. To fill out the table, the permeability constants of the beef erythrocyte for amides were determined by Jacobs' hemolysis method. They are in good agreement with the experiments of Höber and Ørskov (1933). Following Collander and Bärlund (1933), for solutes P is the number of mols crossing one cm.^{2} of membrane in one hour with a concentration difference of one mol per cc., while P_w is the cc. of water crossing one cm.^{2} of membrane with an osmotic pressure difference of one atmosphere.

Two general qualitative principles have emerged from the past studies of permeability: (1) small molecules diffuse into living cells faster than larger ones, (2) lipoid-soluble molecules diffuse into living cells faster than those which are not lipophilic (Höber, 1945). Phascolosoma erythrocytes follow the above rules rather well, as is indicated by their permeability constants in Table I. For example, permeability to alkyl amides increases with increasing hydrocarbon chain length. This probably results from a stronger influence of lipoid solubility than of molecular volume on diffusibility of the molecules. Probably for the same reason esterification of glycerol with acetic acid increases the rate of entrance. On the other hand, in the series of glycols where molecular volume increases without much change in lipoid solubility, the expected decrease in the ease of penetration is seen. Further agreement with the properties of other cells is shown by the increased permeability to 1,3 propandiol versus glycerol, as well as higher permeability to 1,2 propandiol than to its 1,3 isomer.

The results in Table I show that Phascolosoma coelomic cells resemble the other nucleated cells and that they do not possess the special high permeability to both water and urea of beef erythrocytes. In this respect the cells here studied do resemble the erythrocytes of the elasmobranchs, amphibia and reptiles, although only qualitative comparisons can be made with the data of Jacobs *et al.* (1950). In addition, in the beef erythrocyte the effect of lipoid solubility on ascending the homologous series of amides is not apparent from the first member of the series as it is with Arbacia eggs and Phascolosoma erythrocytes.

TABLE I

Permeability constants of selected cells for various substances

Substance	Time in seconds for 50% hemolysis of Phascolosoma "erythrocytes" at 25° C.	$P \times 10^4$ $(\text{cm.} \times \text{hr.}^{-1})$			
	Solute 1 Molar	Phascolosoma	Arbacia eggs	Chara leaf cells	Beef red cells
Formamide	158	59		770†	1760
Acetamide	124	95	345‡	530†	1700
Propionamide	73	374	850‡	1300†	685
Butyramide	53	2460	2200‡	1700†	980
Ethylene Glycol	185	44	240*	430†	58*
Diethylene Glycol	281	21	150*		27*
Triethylene Glycol	637	7.1			12*
Tetraethylene Glycol	1573	2.5			
Urea	552	9.6		40†	6500*
Thiourea	423	12.1		77†	7*
Glycerol	more than 14,400	less than 0.025	3‡	7†	0.7*
Monacetin	1137	3.7		160†	41*
Diacetin	248	26.8		800†	375*
1,3 Propandiol	186	44	260*		36*
1,2 Propandiol	145	69	460*	870†	118*
		P_w $(\text{cm.}^4 \times \text{hr.}^{-1} \times \text{mol.}^{-1})$			
Water	49.2	72	22§ (exosmosis) 18‖ (endosmosis)		400*

* Jacobs (1952); † Collander and Bärlund (1933); ‡ Jacobs and Stewart (1932) § Jacobs (1932); ‖ Lucké, Hartline and McCutcheon (1931).

Experiments with electrolytes throw further light on this question of the general classification of Phascolosoma erythrocytes with respect to their permeability. Their lysis is quite rapid in *M*/2 ammonium acetate, but does not occur in four hours in either *M*/2 ammonium chloride or *M*/2 sodium acetate. The cells therefore appear to be permeable to both ammonia and acetic acid and evidently much less permeable to either sodium ions or chloride ions (Jacobs, 1927). In this respect, therefore, they resemble typical plant and animal cells rather than the vertebrate erythrocyte, in which a special free permeability to anions facilitates the transport of carbon dioxide.

Permeability constants calculated from the hemolysis times in isosmotic solutions of ammonium salts of fatty acids do not have the same significance as those already mentioned because of the complex manner in which these salts enter cells (Jacobs, 1927). However, the hemolysis times shown in Table II, like the results obtained with other cells (Jacobs, 1927; Bouillenne, 1930; Stewart, 1934), indicate that increasing lipoid solubility of the fatty acid goes hand in hand with an increase in the rate of entrance of the salt. It should be noted that in the series: acetate, propionate, butyrate, and valerate, the lysis times are entirely comparable because the pK values of the four acids in question are nearly the same.

TABLE II

50% hemolysis times in M/2 solutions of ammonium salts of fatty acids

Ammonium salt	Time in seconds for 50% hemolysis of Phascolosoma erythrocytes at 25° C.
Formate	1,145
Acetate	388
Propionate	158
Butyrate	93
Valerate	52

The hemerythrin-containing cells of Phascolosoma coelomic fluid resemble the erythrocytes of the lower vertebrates morphologically. Moreover, they contain a high concentration of a red pigment which combines loosely and reversibly with oxygen (Cuénot, 1891), and which they lose by a process analogous to hemolysis of vertebrate erythrocytes. Yet in most instances the present results show that substances enter the Phascolosoma cells more nearly the way they enter Chara cells and Arbacia eggs; in particular, Phascolosoma erythrocytes lack the special free permeability to urea, water, and anions characteristic of the beef erythrocyte.

The author wishes to express his gratitude to Prof. M. H. Jacobs for his help during the course of the work, and to Dr. T. F. Anderson for his aid in preparing the manuscript.

SUMMARY

By virtue of two properties, lysis on reaching a critical volume, and a discoidal shape, the hemerythrin-containing corpuscles of the coelomic fluid of *Phascolosoma gouldi* have proven to be excellent material for the quantitative estimation of permeability constants. The constants evaluated by the method of Jacobs from times of lysis show a general similarity between these cells and unfertilized Arbacia eggs in respect to permeability to water and a series of non-electrolytes. The Phascolosoma cells also resemble Arbacia eggs in their low permeability to both cations and anions.

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